

CLAIMS

1. (currently amended) A method for obtaining purified native ~~purifying~~ Interleukin-4 (IL-4) or muteins thereof comprising: (a) expressing the IL-4 or muteins thereof in a prokaryotic cell thereby forming inclusion bodies containing IL-4 or muteins thereof in said prokaryotic cell; (b) disrupting the prokaryotic cell to release the inclusion bodies; (c) washing ~~separating~~ the inclusion bodies in a washing buffer capable of solubilizing lipids bound to the surface of the inclusion bodies or contained in cell wall fragments from the cell debris; (d) solubilizing the inclusion bodies in a solution that includes a denaturing agent comprising a guanidinium salt, thereby obtaining a guanidine-denatured ~~denaturing the~~ IL-4 or muteins thereof; (e) purifying ~~separating~~ the guanidine-denatured ~~denatured~~ IL-4 or muteins thereof using an immobilized metal chelate affinity chromatography (IMAC) system; and (f) releasing the IL-4 or muteins thereof from the IMAC system; and (g) renaturing the guanidine-denatured IL-4 or muteins thereof in the presence of an artificial chaperone, thereby obtaining ~~the~~ purified native IL-4 or muteins thereof, ~~wherein the step of separating the denatured IL-4 or muteins thereof with the IMAC system provides an average recovery of the IL-4 or muteins thereof of better than 80% and a purity of the IL-4 or muteins thereof of about 90% as estimated by SDS-PAGE analysis.~~

2. (canceled)

3. (previously presented) The method according to claim 2, wherein the washing buffer comprises a non-ionic detergent, an ionic surfactant or a zwitterionic detergent.

4. (previously presented) The method according to claim 2, wherein the washing buffer is a buffer which maintains the pH between 7 and 10.

5. (previously presented) The method according to claim 2, wherein the washing buffer additionally contains a chelating substance.

6. (previously presented) The method according claim 5, wherein the chelating substance is selected from the group consisting of ethylenediamintetraacetic acid (EDTA), ethyleneglycol-O,O' bis-(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), nitriloacetic acid (NTA) and trans-1,2-diamino-cyclohexan-N'N,N',N'-tetraacetic acid (CDTA).

7. (previously presented) The method according to claim 1, wherein the Interleukin-4 is IL-4 R121D Y124D.

8–9. (canceled)

10. (currently amended) The method according to claim 1 9, wherein the artificial chaperone is a cyclic dextrin or linear dextrin.

11. (previously presented) The method according to claim 1, wherein the prokaryotic host is E. coli.

12. (previously presented) The method according to claim 1, wherein the IL-4 is mIL-4 Q116D Y119D.

13–18. (canceled)

19. (previously presented) The method according to claim 3, wherein the zwitterionic detergent is selected from the group consisting of CHAPS, CHAPSO, desoxycholate and the zwittergent series (N-alkyl-N,N-dimethyl-3-ammonio-1-propanesulfonate).

20. (canceled)

21. (new) The method according to claim 1, further comprising the step of releasing the IL-4 or muteins thereof from the IMAC system prior to renaturing the IL-4 or muteins thereof.

22. (new) The method according to claim 1, further comprising the step of releasing the IL-4 or muteins thereof from the IMAC system subsequent to renaturing the IL-4 or muteins thereof.